METHOD FOR FABRICATING A PATTERN IN A MASK ON A SURFACE OF AN OBJECT AND PRODUCT MANUFACTURED THEREBY

[0001] This invention was made with Government support under Grant No. 1 R21 HG02167-01 awarded by the National Institutes of Health. The Government has certain rights in the invention.

[0002] The present application claims benefit under 35 U.S.C. § 119(e) of a provisional U.S. Patent Application of Jon R. Sauer et al. entitled "Charge Sensing and Amplification Device for DNA Sequencing, Serial No. 60/259,584, filed January 4, 2001, the entire content is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention:

[0003] The present invention relates to a system and method for forming nanometer sized openings in a semiconductor structure that can be used for detecting polymers. More particularly, the present relates to a system and method for forming one or more nanometer sized openings in a detecting region of a semiconductor device that is used for identifying the individual mers of long-chain polymers, such as carbohydrates and proteins, as well as individual bases of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), to thus enable sequencing of the strand to be performed.

Description of the Related Art:

[0004] DNA consists of two very long, helical polynucleotide chains coiled around a common axis. The two strands of the double helix run in opposite directions. The two strands are held together by hydrogen bonds between pairs of

bases, consisting of adenine (A), thymine (T), guanine (G), and cytosine (C). Adenine is always paired with thymine, and guanine is always paired with cytosine. Hence, one strand of a double helix is the complement of the other.

[0005] Genetic information is encoded in the precise sequence of bases along a DNA strand. In normal cells, genetic information is passed from DNA to RNA. Most RNA molecules are single stranded but many contain extensive double helical regions that arise from the folding of the chain into hairpin-like structures.

[0006] Mapping the DNA sequence is part of a new era of genetic-based medicine embodied by the Human Genome Project. Through the efforts of this project, one day doctors will be able to tailor treatment to individuals based upon their genetic composition, and possibly even correct genetic flaws before birth. However, to accomplish this task it will be necessary to sequence each individual's DNA. Although the human genome sequence variation is approximately 0.1%, this small variation is critical to understanding a person's predisposition to various ailments. In the near future, it is conceivable that medicine will be "DNA personalized", and a physician will order sequence information just as readily as a cholesterol test is ordered today. Thus, to allow such advances to be in used in everyday life, a faster and more economical method of DNA sequencing is needed.

[0007] One method of performing DNA sequencing is disclosed in U.S. Patent No. 5,653,939, the entire content of which is incorporated herein by reference. This method employs a monolithic array of test sites formed on a substrate, such as a semiconductor substrate. Each test site includes probes which are adapted to bond with a predetermined target molecular structure. The bonding of a molecular structure to the probe at a test site changes the electrical, mechanical and optical properties of the test site. Therefore, when a signal is applied to the test sites, the electrical, mechanical, or optical properties of each test site can be measured to determine which probes have bonded with their respective target molecular structure. However, this method is disadvantageous because the array of test sites is complicated to manufacture, and requires the use of multiple probes for detecting different types of target molecular structures.

[0008] Another method of sequencing is known as gel electrophoresis. Using a polymerase chain reaction (PCR), strands ending with a specific nucleotide are created. The same procedure is repeated for the other three remaining bases. The DNA fragments are separated by gel electrophoresis according to length. The lengths show the distances from the labeled end to the known bases, and if there are no gaps in coverage, the original DNA strand fragment sequence is determined.

[0009] This method of DNA sequencing has many drawbacks associated with it. This technique only allows readings of approximately 500 bases, since a DNA strand containing more bases would "ball" up and not be able to be read properly. Also, as strand length increases, the resolution in the length determination decreases rapidly, which also limits analysis of strands to a length of 500 bases. In addition, gel electrophoresis is very slow and not a workable solution for the task of sequencing the genomes of complex organisms. Furthermore, the preparation before and analysis following electrophoresis is inherently expensive and time consuming. Therefore, a need exists for a faster, consistent and more economical means for DNA sequencing.

[0010] Another approach for sequencing DNA is described in U.S. Patent Nos. 5,795,782 and 6,015,714, the entire contents of which are incorporated herein by reference. In this technique, two pools of liquid are separated by a biological membrane with an alpha hemolysin pore. As the DNA traverses the membrane, an ionic current through the pore is blocked. Experiments have shown that the length of time during which the ionic current through the pore is blocked is proportional to the length of the DNA fragment. In addition, the amount of blockage and the velocity depend upon which bases are in the narrowest portion of the pore. Thus, there is the potential to determine the base sequence from these phenomena.

[0011] Among the problems with this technique are that individual nucleotides cannot, as yet, be distinguished. Also, the spatial orientation of the individual nucleotides is difficult to discern. Further, the electrodes measuring the charge flow are a considerable distance from the pore, which adversely affects the accuracy of the measurements. This is largely because of the inherent capacitance of the current-sensing electrodes and the large statistical variation in sensing the small amounts of

current. Furthermore, the inherent shot noise and other noise sources distort the signal, incurring additional error. Therefore, a need exists for a more sensitive detection system which discriminates among the bases as they pass through the sequencer.

SUMMARY OF THE INVENTION

[0012] An object of the present invention is to provide a system and method for forming nanometer sized openings in an object, such as a semiconductor structure to enable the structure to be used for accurately detecting polymers.

[0013] Another object of the present invention is to provide a system and method for forming one or more nanometer sized openings in a detecting region of a semiconductor device that is used for identifying the individual mers of long-chain polymers, such as carbohydrates and proteins, as well as individual bases of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), to thus enable sequencing of the strand to be performed.

[0014] These and other objects of the invention are substantially achieved by providing a system and method which employ the operations of positioning a mask pattern including a plurality of mask lines at a first orientation with respect to a mask on the object, such as a semiconductor, and imposing the mask pattern on the mask to create first mask lines extending in a first direction along the mask. The system and method then move the mask pattern to a second orientation with respect to the mask on the semiconductor, and impose the mask pattern on the mask to create second mask lines extending in a second direction along the mask which is transverse of the first direction and overlapping the first mask lines, such that said surface of said object is exposed at areas of said mask between said first and second mask. The system and method further remove portions of the semiconductor at the removed portions of the mask to form the openings in the semiconductor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] These and other objects, advantages and novel features of the invention will be more readily appreciated from the following detailed description when read in conjunction with the accompanying drawings, in which:

[0016] Fig. 1 illustrates a system for performing DNA or RNA sequencing comprising a DNA or RNA sequencer constructed in accordance with an embodiment of the present invention;

[0017] Fig. 2 illustrates a top view of the DNA or RNA sequencer shown in Figure 1;

[0018] Fig. 3 is a graph showing an example of the waveform representing the current detected by a current detector in the system shown in Figure 1 as the adenine (A), thymine (T), guanine (G), and cytosine (C) bases of a DNA or RNA sequence pass through the DNA or RNA sequencer;

[0019] Fig. 4 illustrates a cross-sectional view of a silicon-on-insulator (SOI) substrate from which a DNA or RNA sequencer as shown in Figure 1 is fabricated in accordance with an embodiment of the present invention;

[0020] Fig. 5 illustrates a cross-sectional view of the SOI substrate shown in Figure 5 having shallow and deep n-type regions formed in the silicon layer, and a portion of the substrate etched away;

[0021] Fig. 6 illustrates a cross-sectional view of the SOI substrate shown in Figure 5 in which a portion of the insulator has been etched away and another shallow n-type region has been formed in the silicon layer;

[0022] Figs. 7A and 7B are images of opening patterns formed in a semiconductor structure using a cross-line technique in accordance with an embodiment of the present invention;

[0023] Fig. 8 is an image of a photograph of a pattern of etched lines formed in a semiconductor structure using the cross-line technique as shown in Figs. 7A and 7B in accordance with an embodiment of the present invention;

[0024] Fig. 9 illustrates a detailed cross-sectional view of an exemplary configuration of the opening in the SOI substrate;

[0025] Fig. 10 illustrates a top view of the opening shown in Fig. 9;

[0026] Fig. 11 illustrates a cross-sectional view of the SOI substrate having an opening etched therethrough;

[0027] Fig. 12 illustrates a top view of the SOI substrate as shown in Fig. 11;

[0028] Fig. 13 illustrates a cross-sectional view of the SOI substrate shown in Fig. 11 having an oxidation layer formed on the silicon layer and on the walls forming the opening therein;

[0029] Fig. 14 illustrates a top view of the SOI substrate as shown in Fig. 13;

[0030] Fig. 15 illustrates a detailed cross-sectional view of the SOI substrate shown in Fig. 13 having an oxidation layer formed on the silicon layer and on the walls forming the opening therein;

[0031] Fig. 16 illustrates a top view of the SOI substrate shown in Fig. 15;

[0032] Fig. 17 illustrates a cross-sectional view of the SOI substrate as shown in Fig. 13 having holes etched in the oxidation layer and metal contacts formed over the holes to contact the shallow and deep n-type regions, respectively;

[0033] Fig. 18 illustrates a cross-sectional view of the DNA or RNA sequencer shown in Fig. 1 having been fabricated in accordance with the manufacturing steps shown in Figs. 4-17; and

[0034] Fig. 19 illustrates a top view of a DNA or RNA sequencer having multiple detectors formed by multiple n-type regions according to another embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0035] Figs. 1 and 2 illustrate a system 100 for detecting the presence of a polymer, such as DNA or RNA, a protein or carbohydrate, or a long chain polymer such as petroleum, and, more preferably, for identifying the individual mers of the polymer or long chain polymer, as well as the length of the polymer or long chain polymer. The system 100 is preferably adaptable for performing sequencing of nucleic acids, such as DNA or RNA sequencing, according to an embodiment of the

present invention. Accordingly, for purposes of this description, the system 100 will be discussed in relation to nucleic acid sequencing.

[0036] The system 100 includes a nucleic acid sequencing device 102 which, as described in more detail below, is a semiconductor device. Specifically, the nucleic acid sequencing device 102 resembles a field-effect transistor, such as a MOSFET, in that it includes two doped regions, a drain region 104 and a source region 106. However, unlike a MOSFET, the nucleic acid sequencing device does not include a gate region for reasons discussed below.

[0037] The nucleic acid sequencing device 102 is disposed in a container 108 that includes a liquid 110 such as water, gel, a buffer solution such as KCL, or any other suitable solution. It is important to note that the solution 110 can be an insulating medium, such as oil, or any other suitable insulating medium. In addition, the container 108 does not need to include a medium such as a liquid. Rather, the container 108 can be sealed and evacuated to create a vacuum in which nucleic acid sequencing device 102 is disposed. Also, although Fig. 1 shows only a single nucleic acid sequencing device 102 in the container 108 for exemplary purposes, the container can include multiple nucleic acid sequencing devices 102 for performing multiple DNA sequencing measurements in parallel.

[0038] The liquid 110 or other medium or vacuum in container 108 includes the nucleic acid strands or portions of nucleic acid strands 111 to be sequenced by nucleic acid sequencing device 102. As further shown, voltage source 112, such as a direct current voltage source, is coupled in series with a current meter 114 by leads 116 across drain and source regions 104 and 106, respectively. In this example, the positive lead of voltage source 112 is coupled to the drain region 104 while the negative lead of voltage source 112 is coupled via the current meter 114 to source region 106.

[0039] The voltage potential applied across drain and source regions 104 and 106 of nucleic acid sequencing device 102 can be small, for example, about 100 mV, which is sufficient to create a gradient across drain and source regions 104 and 106, to draw the nucleic acid strands into opening 118 of the nucleic acid sequencing device

102. That is, the nucleic acid strands 111 move through the opening 118 because of the local gradient. Alternatively or in addition, the liquid can include an ionic solution. In this event, the local gradient causes the ions in the solution to flow through the opening 118, which assists the nucleic acid strands 111, such as DNA or RNA, to move through the opening 118 as well.

[0040] Additional electrodes 113 and 115 positioned in the medium 110 and connected to additional voltage sources 117 and 121 would further facilitate the movement of the nucleic acid strands toward the opening 118. In other words, the external electrodes 113 and 115 are used to apply an electric field within the medium 110. This field causes all of the charged particles, including the nucleic acid strand 111, to flow either toward the opening 118 or away from the opening 118. Thus electrodes 113 and 115 are used as a means to steer the nucleic acid strands 111 into or out of the opening 118. In order to connect voltage sources 112 and 117 to the nucleic acid sequencer 102, metal contacts 123 are coupled to the n-type doped region 128 and 130, described in more detail below. The electrodes 113 and 115 could also provide a high frequency voltage which is superimposed on the DC voltage by an alternating voltage source 125. This high frequency voltage, which can have a frequency in the radio frequency range, such as the megahertz range (e.g., 10 MHz), causes the nucleic acid strand 111 and ions to oscillate. This oscillation makes passage of the nucleic acid strand 111 through the opening 118 smoother, in a manner similar to shaking a salt shaker to enable the salt grains to pass through the openings in the shaker. Alternatively, a device 127, such as an acoustic wave generator, can be disposed in the liquid 110 or at any other suitable location, and is controlled to send sonic vibrations through the device 102 to provide a similar mechanical shaking function.

[0041] As can be appreciated by one skilled in the art, the nucleic acid strands each include different combinations of bases A, C, G and T, which each contain a particular magnitude and polarity of ionic charge. The charge gradient between drain and source regions 104 and 106, or otherwise across the opening 118, will thus cause the charged nucleic acid strands to traverse the opening 118. Alternatively, another

voltage source (not shown) can be used to create a difference in voltage potential between the opening 118 and the liquid. Also, a pressure differential can be applied across the opening 118 to control the flow of the DNA independent from the voltage applied between the source and drain 104 and 106.

[0042] In addition, the sequencing device 102 can attract the nucleic acid strands to the opening 118 by applying a positive voltage to the medium 110 relative to the voltage source 112. Furthermore, the nucleic acid strands in the medium 110 can be pushed in and out of the opening 118 and be analyzed multiple times by reversing the polarity across drain and source regions 104 and 106, respectively.

[0043] As described in more detail below, the opening 118 is configured to have a diameter within the nanometer range, for example, within the range of about 1 nm to about 10 nm. Therefore, only one DNA strand can pass through opening 118 at any given time. As a DNA strand passes through opening 118, the sequence of bases induce image charges which form a channel 119 between the drain and source regions 104 and 106 that extends vertically along the walls of the device defining opening 118. As a voltage is applied between the source 136 and drain 128 by means of the voltage source 112, these image charges in the channel flow from source to drain, resulting in a current flow which can be detected by the current meter 114. The current exists in the channel as long as the charge is present in the opening 118, and thus the device current detected by the current meter 114 is much larger than the current associated with the moving charge. For example, a singly charged ion passing through the opening 118 in one microsecond accounts for an ion current of 0.16 pA and a device current of 160 nA.

[0044] Alternatively, the bases induce a charge variation in channel 119, leading to a current variation as detected by current meter 114. Any variation of the ion flow through the opening due to the presence of the DNA strand would also cause a variation to the image charge in the channel 119 and results in a current variation as detected by current meter 114. That is, the device current measured by current meter 114 will diminish from, for example, 80 μA to 4 μA. as the DNA strand 111 passes through opening 118.

[0045] Each different type of bases A, C, G, and T induces a current having a particular magnitude and waveform representative of the particular charge associated with its respective type of bases. In other words, an A type base will induce a current in a channel between the drain and source regions of the nucleic acid sequencing device 102 having a magnitude and waveform indicative of the A type base. Similarly, the C, T and G bases will each induce a current having a particular magnitude and waveform.

[0046] An example of a waveform of the detected current is shown in Fig. 3, which symbolically illustrates the shape, magnitude, and time resolution of the expected signals generated by the presence of the A, C, G and T bases. The magnitude of current is typically in the microampere (μA) range, which is a multiplication factor of 10^6 greater than the ion current flowing through the opening 118, which is in the picoampere range. A calculation of the electrostatic potential of the individual bases shows the complementary distribution of charges that lead to the hydrogen bonding. For example, the T-A and C-G pairs have similar distributions when paired viewed from the outside, but, when unpaired, as would be the case when analyzing single-stranded DNA, the surfaces where the hydrogen bonding occurs are distinctive. The larger A and G bases are roughly complementary (positive and negative reversed) on the hydrogen bonding surface with similar behavior for the smaller T and C bases.

[0047] Accordingly, as the DNA strand passes through opening 118, the sequence of bases in the strand can be detected and thus ascertained by interpreting the waveform and magnitude of the induced current detected by current meter 114. The system 100 therefore enables DNA sequencing to be performed in a very accurate and efficient manner.

[0048] Since the velocity of the electrons in the channel 119 is much larger than the velocity of the ions passing through the opening, the drain current is also much larger than the ion current through the opening 118. For an ion velocity of 1 cm/s and an electron velocity of 10^6 cm/s, an amplification of 1 million can be obtained.

[0049] Also, the presence of a DNA molecule can be detected by monitoring the current I_P through the opening 118. That is, the current I_P through the opening reduces from 80 pA to 4 pA when a DNA molecule passes through the opening. This corresponds to 25 electronic charges per microsecond as the molecule passes through the opening.

[0050] Measurement of the device current rather than the current through the opening has the following advantages. The device current is much larger and therefore easier to measure. The larger current allows an accurate measurement over a short time interval, thereby measuring the charge associated with a single DNA base located between the two n-type regions. In comparison, the measurement of the current through the opening has a limited bandwidth, limited by the shot-noise associated with the random movement of charge through the opening 118. For example, measuring a 1pA current with a bandwidth of 10 MHz yields an equivalent noise current of 3.2 pA. Also, the device current can be measured even if the liquids on both sides of the opening 118 are not electrically isolated. That is, as discussed above, the sequencing device 102 is immersed in a single container of liquid. Multiple sequencers 102 can thus be immersed in a single container of liquid, to enable multiple current measurements to be performed in parallel. Furthermore, the nanometer-sized opening 118 can be replaced by any other structure or method which brings the DNA molecule in close proximity to the two n-type regions, as discussed in more detail below.

[0051] The preferred method of fabricating a nucleic acid sequencing device 102 will now be described with reference to Figs. 4-16. As shown in Fig,. 4, the fabrication process begins with a wafer 120, such as a silicon-on-insulator (SOI) substrate comprising a silicon substrate 122, a silicon dioxide (SiO_2) layer 124, and a thin layer of p-type silicon 126. In this example, the silicon substrate 122 has a thickness within the range of about 300 µm to about 600 µm, the silicon dioxide layer 124 has a thickness within the range of about 200 to 6400 nm, and the p-type silicon layer 126 has a thickness of about 1 µm or less (e.g., within a range of about 10 nm to about 1000 nm).

[0052] As shown in Fig. 5, a doped n-type region 128 is created in the p-type silicon layer 126 by ion implantation, and annealing or diffusion of an n-type dopant, such as arsenic, phosphorous or the like. As illustrated, the n-type region 128 is a shallow region which does not pass entirely through p-type silicon 126. A deep n-type region 130 is also created in the p-type silicon 126 as illustrated in Fig. 5. The deep n-type region 130 passes all the way through the p-type silicon 126 to silicon dioxide 124 and is created by known methods, such as diffusion, or ion implantation and annealing of an n-type material which can be identical or similar to the n-type material used to create n-type region 128. As further illustrated in Fig. 5, the silicon substrate 122 is etched along its (111) plane by known etching methods, such as etching in potassium hydroxide (KOH) or the like. The back of the substrate 112 can also be etched with a teflon jig. As illustrated, the etching process etches away a central portion of silicon substrate 122 down to the silicon dioxide 124 to create an opening 132 in the silicon substrate 122.

[0053] As shown in Fig. 6, the portion of the silicon dioxide 124 exposed in opening 132 is etched away by conventional etching methods, such as etching in hydrofluoric acid, reactive etching or the like. Another shallow n-type region 124 is created in the area of the p-type silicon 126 exposed at opening 132 by known methods, such implantation or diffusion of an n-type material identical or similar to those used to create n-type regions 128 and 130.

[0054] Opening 118 (see Figures 1 and 2) is then formed through the n-type region 128, p-type silicon 126 and bottom n-type region 134 as shown, for example, in Figs. 7A, 7B and 8. The opening 118 can first be made as one of a plurality of well-defined square holes 200 in a silicon wafer 202 as shown in Figs. 7A and 7B. A masking material 204, such as SiO_2, is deposited on top of the silicon wafer 202. To form these holes, a series of lines with the appropriate width and spacing is defined in a pattern (not shown), and the pattern is then transferred into the masking material 204 on the surface of the silicon wafer 202 as shown in Fig. 8, as will now be described.

[0055] Specifically, a photosensitive layer (not shown) is deposited on top of the silicon dioxide masking material 204, and the pattern is used to expose and develop a set of lines into the photosensitive layer. The exposed portion of the photosensitive layer is then removed, and the exposed masking material 204 is then partially etched, for example, using hydrofluoric acid, with the remaining portions of the photosensitive layer acting as a mask. Accordingly, a pattern as shown in Fig. 8 having lines 206 is etched into the masking material 204. Generally, the lines 206 have a depth only partially into the silicon dioxide masking material 204, but not down to the surface of the silicon wafer 202.

[0056] The photosensitive layer is then removed and replaced with a fresh photosensitive layer. The same pattern used to form the lines 206 shown in Fig. 8 is then rotated by 90°, and the exposing, developing and etching steps discussed above are repeated to remove the exposed masking material 204. It is noted that the pattern need not be rotated exactly by 90°, but can be rotated to any suitable angle transverse to the lines 206. It is further noted that because the etching is performed to only a partial depth of the masking material 204, only the regions 200 (See Fig. 7A) defined by the overlapping areas between the two sets of lines is removed down to the surface of the silicon wafer 202 during etching. That is, the portions of the lines 206 which are now covered by the remaining portions of the photosensitive layer are not further etched during the etching process. However, the portions of the lines 206 that are exposed will be further etched to a depth which exposes the surface of the silicon wafer 202. Also, even though other exposed portions of the masking material 204 will be etched by this second etching process, those portions were covered by the first photosensitive layer used during the first etching process to form lines 206. Hence, the partial etching performed during this second etching process will not etch those portions to a depth sufficient to expose the silicon wafer 202. Accordingly, the pattern shown in Fig. 7A results.

[0057] The silicon dioxide layer masking material 204 can now be used as a masking layer to etch the silicon wafer 202. A different chemical is used, namely potassium hydroxide dissolved in water. This chemical is known to etch silicon but

does not etch silicon dioxide. Moreover, this etch will expose the (111) crystal planes in the silicon resulting in a hole shaped like an inverted pyramid as shown in Fig. 7B.

[0058] This process leads to a much better edge definition of the holes compared to defining the holes in a single lithographic step, as can be appreciated from the pattern shown in Fig. 27A having openings 200. The pattern shown in Figs. 7A and 7B was made with the technique described above using a line pattern with 3 μm width and 3 μm spacing. The resulting etch mask was then used to etch the pits in the silicon 202 using potassium hydroxide (KOH).

[0059] A further reduction of the line width can be achieved using electron-beam lithography. For example, electron-beam lithography using a Phillips 515 scanning electron microscope (SEM) can produce a line pattern with a 100 nm width. Polymethyl methacrylite (PMMA) can be used as an electron resist and developed with methyl iso butyl ketone/ isopropyl alcohol (MIBK/IPA) to achieve the pattern 204 shown in Figs. 7A and 7B having openings 206. As illustrated, the lines are well defined and are limited by the spot size of the beam used in the electron-beam lithography. The beginning of each line is rounded since a single exposure with a gaussian beam has been used. This rounding can be eliminated by using the crossed-line lithography technique described with regard to Figs. 7A and 7B. The PMMA can also be used as an etch mask to successfully transfer the pattern into a thin SiO_2 layer as shown.

[0060] Accordingly, an opening 118 can be fabricated on (100) silicon membranes by combining state-of-the-art electron beam lithography with two well-known size reduction techniques discussed above. A scanning transmission electron microscope (STEM) can be used to define 10 nm lines in PMMA. Crossed lines can be used to create 10 nm square holes in a SiO_2 mask. KOH etching can be used to etch V-shaped pits, providing a 2-4 nm opening on the other side of the silicon membrane. Alternatively, reactive ion etching (RIE) using Freon 14 (CF_4), optical lithography, electron-beam lithography or any other fine-line lithography, can be used which results in an opening having a diameter of about 10 nm.

[0061] Although for illustration purposes Figs. 1, 2 and 11-18 show opening 118 as being a cylindrically-shaped opening, the opening 118 formed according to the techniques described above has a funnel shape as shown, for example, in Figs. 9 and 10. This funnel-shaped opening 118 is created by performing V-groove etching of the (100) p-type silicon layer 126 using potassium hydroxide (KOH), which results in V-shaped grooves formed along the (111) planes 138 of the p-type silicon 126. The V-shaped or funnel-shaped opening, as shown explicitly in Fig. 10, facilitates movement of a DNA strand through opening 118, and minimizes the possibility that the DNA strand will become balled up upon itself and thus have difficulty passing through opening 118. Oxidation and V-groove etching can be combined to yield even smaller openings. Additionally, anodic oxidation can be used instead of thermal oxidation, as described above. Anodic oxidation has the additional advantage of allowing for monitoring of the opening size during oxidation so that the process can be stopped when the optimum opening size is achieved.

[0062] Specifically, the opening 118 should be small enough to allow only one molecule of the DNA strand 111 to pass through at one time. Electron-beam lithography can yield an opening 118 as small as 10 nm, but even smaller openings are needed. Oxidation of the silicon and V-groove etching as described above can be used to further reduce the opening to the desired size of 1-2 nm. Oxidation of silicon is known to yield silicon dioxide with a volume which is about twice that of the silicon consumed during the oxidation. Oxidation of a small opening 118 will result in a gradually reduced opening size, thereby providing the desired opening size V-groove etching of (100) oriented silicon using KOH results in V-grooves formed by (111) planes. KOH etching through a square SiO_2 or Si_3N_4 mask results in a funnel shaped opening with a square cross-section. Etching through the thin silicon layer results in an opening 118 on the other side, which is considerable smaller in size.

[0063] Oxidation and V-groove etching can also be combined to yield even smaller openings 118. Anodic oxidation can be used instead of thermal oxidation, which has the additional advantage of enabling the size of the opening 118 to be

monitored during the oxidation and the oxidation can be stopped when the appropriate size of the opening 118 is obtained.

[0064] The opening 118 will be further reduced in size by thermal oxidation of the silicon as it results in an oxide, which has about twice the volume of the oxidized silicon. This oxidation also provides the gate oxide, as discussed above.

[0065] That is, as shown in Fig. 13, the diameter of the opening can be further decreased by oxidizing the silicon, thus forming a silicon dioxide layer 136 over the p-type silicon layer 126 and the walls forming opening 118. This oxidation can be formed by thermal oxidation of the silicon in an oxygen atmosphere at 800-1000°C, for example. As shown in detail in Figs. 14-16, the resulting oxide has a volume larger than the silicon consumed during the oxidation process, which further narrows the diameter of opening 118. It is desirable if the diameter of opening 118 can be as small as 1 nm.

[0066] Turning now to Fig. 17, holes 140 are etched into the silicon dioxide 136 to expose n-type region 128 and n-type region 130. Metal contacts 142 are then deposited onto silicon dioxide layer 136 and into holes 140 to contact the respective n-type regions 128 and 130. An insulator 144 is then deposited over metal contacts 142 as shown in Fig. 18, thus resulting in device 102 as shown in Fig. 1.

[0067] As further shown in Fig. 1, a portion of insulator 144 can be removed so that leads 116 can be connected to the n-type regions 128 and 130, which thus form the drain regions 104 and source 106, respectively. An additional insulator 146 is deposited over insulator 144 to seal the openings through which leads 116 extend to contact n-type regions 128 and 130. The completed device 102 can then be operated to perform the DNA sequencing as discussed above.

[0068] To identify the bases of the DNA molecule, it is desirable to measure a single electronic charge. If the sequencing device 102 is made to have a length and width of 0.1 by 0.1 μm, and the thickness of the silicon dioxide layer is 0.1 μm along the walls of the opening 118, a capacitance of 0.35 fF, a voltage variation of 0.45 mV, a device transconductance of 1 mS and a current variation of 0.5 nA are realized. Accordingly, a sequencing device 102 having these dimensions and characteristics

can be used to detect a single electronic charge, or a portion of the charge, passively, meaning without any chemical reaction, binding or any other similar reaction. The sequencing device 102 can further be reduced in size to obtain a sufficient special resolution to distinguish between different nucleotides. The sequencing device 102 is preferably made smaller to have an improved charge sensing capability. For example, the width of the sequencing device can be 10 nm, the length can be 10 nm, and the opening 118 can have a diameter of 1 nm.

[0069] Additional embodiments of the device 102 can also be fabricated. For example, Fig. 19 illustrates a top view of a nucleic acid sequencing device according to another embodiment of the present invention. In this embodiment, the steps described above with regard to Figs. 4 through 18 are performed to form the n-type regions which ultimately form the drain and source regions. However, in this embodiment, the n-type region 128 shown, for example, in Fig. 5, is formed as four separate n-type regions, 150 in a p-type silicon layer similar to p-type silicon layer 126 described above. A silicon dioxide layer 152 covers the p-type silicon layer into which n-type regions 150 have been created. Holes 156 are etched into silicon dioxide layer 152 so that metal contacts 158 that are deposited on silicon dioxide layer 152 can contact n-type regions 150. By detecting current flowing between the four drain regions formed by n-type regions 150 and the source region (not shown), the spatial orientation of the bases on the DNA strand passing through opening 152 can be detected.

[0070] In addition, any of the DNA sequencers described above (e.g., sequencing device 102) can contain an alternative to the barrier (e.g., oxide layer 136) between the semiconductor channel (e.g., channel 119 in sequencing device 102 shown in Fig. 1) and the medium containing the DNA molecules (e.g., liquid 110 shown in Fig. 1). For example, the oxide barrier 136 can be removed, which still leaves a potential barrier between the semiconductor and the medium. The oxide layer 136 can be replaced by a wider bandgap semiconductor doped with donors and/or acceptors. The oxide layer 136 can also be replaced by an undoped wider bandgap semiconductor layer.

[0071] Furthermore, the oxide layer 136 can be replaced with an oxide containing one or more silicon nanocrystals. The operation of a sequencing device 102 with this type of a barrier is somewhat different compared than that of a sequencing device 102 with an oxide layer 136. That is, rather than directly creating an image charge in the semiconductor channel 119, the charge of the individual nucleotides polarizes the nanocrystal in the barrier. This polarization of the nanocrystal creates an image charge in the semiconductor channel 119. The sensitivity of the sequencing device 102 will be further enhanced as electrons tunnel from the nucleotide into the nanocrystal. The charge accumulated in the nanocrystal can be removed after the measurement (e.g., current reading by current meter 114) by applying a short voltage pulse across the drain and source of the sequencing device 102.

[0072] As discussed above, the size of the opening in the sequencing device (e.g., opening 118 in sequencing device 102) can be varied over a large range. However for proper operation, the opening 118 must be small enough so that the DNA is in close proximity to the charge sensor and large enough so that the DNA can traverse the opening. Since the diameter of a single stranded DNA molecule equals about 1.5 nm, the opening should be between 1 and 3 nm for optimal sensing. Larger openings may result in reduced signal to noise ratio, but would provide a larger ion flow through the opening 118.

[0073] In addition, an electric field could be imposed along the longer axis of the opening 118 to align the base intrinsic dipole moment of the nucleotide with the field. For example, the dipole moment of Cytosine is 6.44 Debye, the dipole moment of Thymine is 4.50 Debye, the dipole moment of Adenine is 2.66 Debye and the dipole moment of Guanine is 6.88 Debye. If the field is strong enough, it can stretch the base (nucleotide) along the dipole moment, thus bringing the charges on the base nearer to the sensors and increasing sensitivity. These techniques will thus make the data much easier to interpret, and will increase the signal used to discriminate between bases.

[0074] All of the devices described above can also be modified in other ways. For example, the SiO_2 oxide layer can be converted to Si_3N_4 in a nitrous oxide (NO)

ambient for use in alkaline solutions. Furthermore, since DNA molecules 111 are negatively charged, the molecules 111 can be attracted to the opening 118 by using electrodes, such as electrodes 113 and 115, to apply a positive voltage to the liquid 110 relative to the source of the device.

[0075] As discussed above, a gel can be used in place of liquid 110 to contain the DNA molecules. The use of a gel will slow down the motion of the ions and further improve the signal to noise ratio. Furthermore, a pressure differential can be applied across the opening to control the flow independent from the applied voltage between source and drain.

[0076] Double stranded DNA can be analyzed as well. Even though double stranded DNA is a neutral molecule, since the molecule contains charge, the nucleotides can be identified by charge sensing. In addition, other molecules, for example, a fluorescent dye such as Hoechst dye, can be attached to single stranded DNA to enhance/modify the stiffness of the molecule thereby facilitating the insertion of the molecule into the nanometer-sized opening. Furthermore, since the above devices can by used to analyze generally any types of individual polymers, they can be used in industries dealing with polymers such as the petroleum industry, pharmaceutical industry and synthetic fiber industry, to name a few.

[0077] Although only a few exemplary embodiments of the present invention have been described in detail above, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the following claims.